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Studies on gramicidin channels

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Abstract: We have investigated the rate of incorporation of gramicidin in asolectin (Soyabean phospholipid) vesicles prepared by sonication. The vesicles contain trapped pyranine, a pH sensitive fluorescent dye that is used to monitor the influx of protons. A pH gradient is created by externally added HCl (ΔpH = 0.5 units) and the decrease in fluorescence of the trapped pyranine is monitored. The kinetics of the incorporation of gramicidin is obtained graphically. Our results are inconsistent with the assumption that the gramicidin is distributed on the vesicles according to Poisson distribution. It also appears that one gramicidin dimer can effectively cause the pH gradient of several vesicles to collapse. The simplest assumption consistent with this observation is that active channels are exchangeable between vesicles. Graphical studies show that kinetically two regions can be distinguished – one corresponding to low concentration of gramicidin and the other corresponding to the high concentration. It is proposed that different kinetic processes are responsible in these two regions.

Keywords: Gramicidin channels, vesicles/liposomes, fluorescence studies, kinetics.

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1. Introduction

The ability of the cell to maintain complex internal environment is due to the strong and selective barrier action of cellular membranes to hydrophilic molecules. The permeability of hydrophobic molecules are thermodynamically favourable due to the presence of lipid matrix. Addition of ionophores makes the membrane leaky to certain ions. Model membranes like planar lipid bilayers and liposomes are generally more permeable to hydrogen/hydroxide ions than other cations like sodium and potassium. Two alternative mechanisms have been suggested for this anomalous proton flux across lipid bilayers (Gutknecht and Walter 1981, Gutknecht 1987). The first one is that the flux is maintained by traces of weak acid protonophores in all lipid bilayers arising as a product of oxidation. The other is due to the formation of hydrogen bonded chains of water molecules within

bilayers. Electrical studies on black lipid membranes have shown that cation permeability through channel forming ionophore is much faster than that mediated by a mobile carrier, e.g., valinomycin. Membrane-bound gramicidin exhibits a much less pronounced cation specificity than valinomycin. Gramicidin is a hydrophobic linear polypeptide antibiotic consisting of 15 amino acids with alternating L and D configuration. In membranes it forms helical dimers that are conducting channels for small monovalent cations (Wallace 1986).

2. Materials and methods

In the present study we have used liposomes as the model system for the transport studies because of its large surface area and its size can be controlled. They can

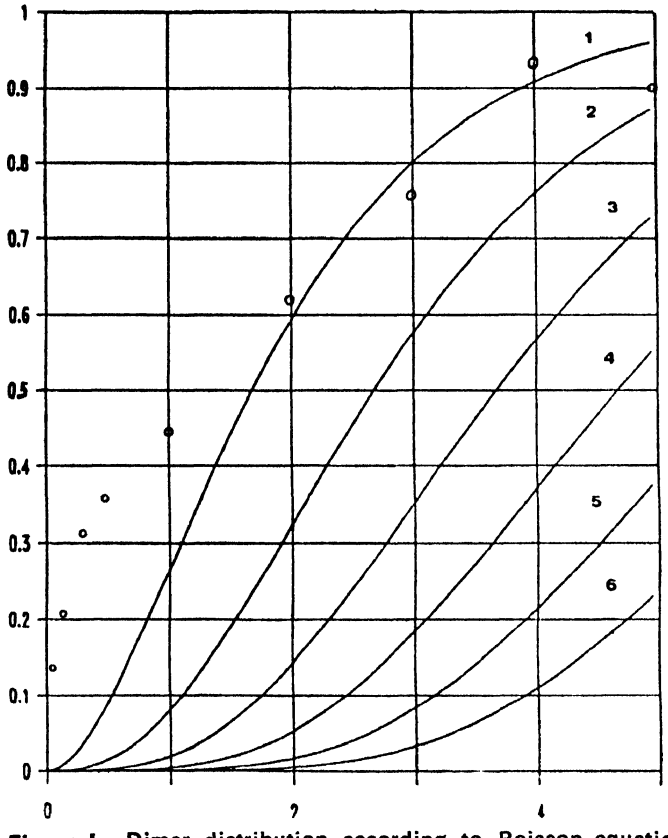

Figure 1. Dimer distribution according to Poisson equation. O: Experimentally observed points; X axis: Ratio of gramicidin dimer/vesicle; Y axis: Fraction of vesicles having more than one dimer.
1. Number of vesicles having more than one dimer. 2. Number of vesicles having more than two dimers. 3. Number of vesicles having more than three dimers. 4. Number of vesicles having more than four dimers and so on.

be prepared from a variety of phospholipids and lipid mixtures. Asolectin (acetone washed; Kagawa 1972) (Sigma catalogue no: p-3644) and egg phospholipid

(used without purification) (Sigma catalogue no : p-9671) are used as the starting materials. Asolectin was sonicated in a buffer which consists of 15 mM KH_2PO_4, 150 mM KCl, 1 mM pyranine, pH 7.5 at a concentration of 50 mg/mL until the suspension became transparent to visible light (O.D. = 0.35 at 1 cm path). The preparation was passed through Sephadex G-50 column to remove any external

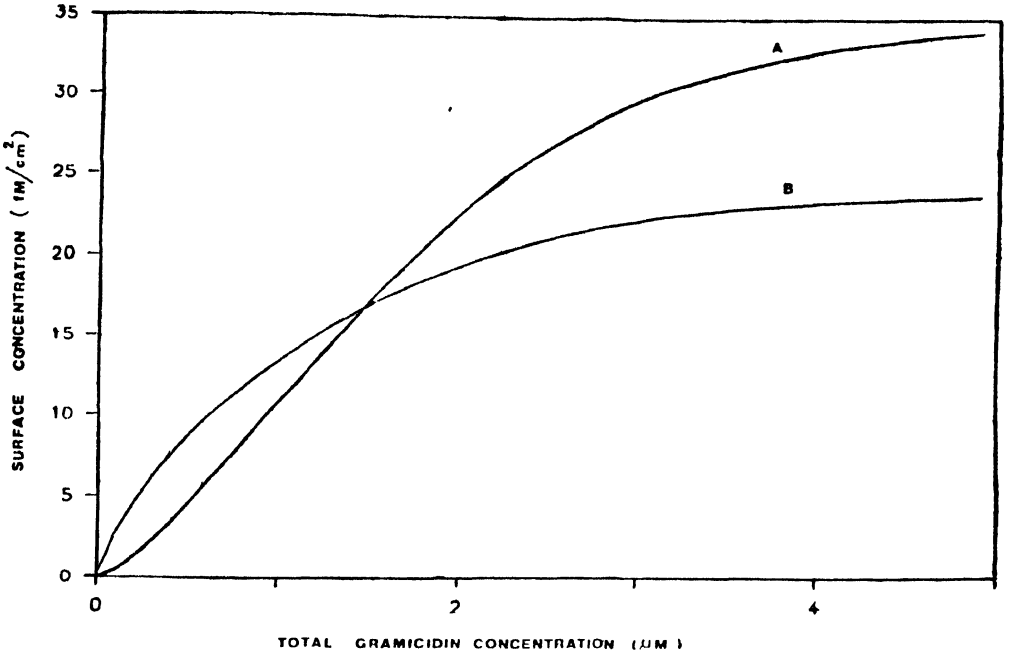

Figure 2. A. dimer; B. monomer.

pyranine. Thus obtained liposomal fraction was diluted to a final concentration of 2.5 mM phospholipid (M. W. taken as 1000) and were allowed to equilibrate for at least 1 hr in dark. 1.0 mL of liposomal suspension was taken into a spectrofluorimeter cuvette and the excitation and emission wave lengths were fixed at 466 nm and 535 nm. A pH gradient of 0.5 pH units was created across the liposomes with the addition of known aliquots of standard HCl (0.1 N stock). The decay of pH gradient was slow in the absence of any ionophore. Addition of gramicidin leads to an immediate flux of protons which was monitored by the decrease in fluorescence of pyranine. The initial slopes in the fluorescence curve were measured manually. Liposomes with different mole percent of cholesterol was also used to check the influence of fluidity of membrane on the rate of incorporation of gramicidin.

3. Results

The approximate radii of the vesicles, as reported in the literature, is assumed to be in range of 20-40 nm (Knight 1981). The internal volume of each liposomes